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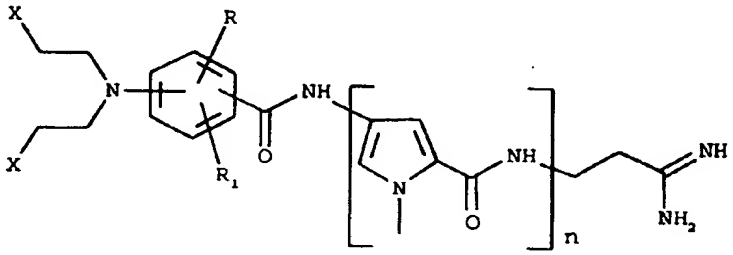
(54) Title: BIS-(2-HALOETHYL)AMINOPHENYL SUBSTITUTED DISTAMYCIN DERIVATIVES AS ANTITUMOR AND ANTIVIRAL AGENTS



(I)

(57) Abstract

Novel antitumor and antiviral agents of formula (I) wherein n is 2, 3 or 4; one of R and R_1 is hydrogen, C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy and the other is independently CF_3, C_1-C_4 alkyl or C_1-C_4 alkoxy; and X is halogen; and the salts thereof are disclosed.

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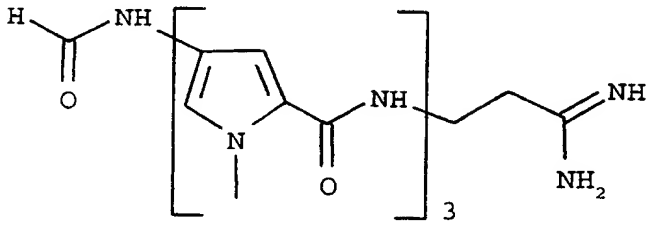
BIS-(2-HALOETHYL)AMINOPHENYL SUBSTITUTED DISTAMYCIN DERIVATIVES AS ANTITUMOR AND ANTIVIRAL AGENTS

The present invention refers to novel antitumor alkylating and antiviral agents related to the known antibiotic distamycin A.



(distamycin A)

which belongs to the family of the pyrroleamidine antibiotics and is reported to interact reversibly and selectively with DNA-AT sequences interfering with both replication and transcription [Nature 203, 1064 (1964); FEBS Letters 7 (1970) 90; Prog. Nucleic Acids Res.Mol.Biol., 15, 285 (1975)].

DE-A-1795539 describes the preparation of distamycin derivatives in which the formyl group of distamycin is replaced by hydrogen or the acid residue of an organic C_1-C_4 aliphatic acid or of cyclopentylpropionic acid.

EP-B-246868 describes distamycin A analogs in which the distamycin formyl group is substituted by aromatic, alicyclic or heterocyclic moieties bearing alkylating groups.

It has now been found that a selected class of compounds falling within the general chemical formula of EP-B-246868 has more valuable biological properties than the related prior art compounds.

Accordingly the present invention provides new site specific nitrogen mustards, a process for their preparation, pharmaceutical compositions containing them and their use in

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groups are each other preferably in the meta or para positions.

R and R_1 can be on any of the free carbon atoms of the phenyl ring, not on the same carbon atom of course. Preferably one of R and R_1 is hydrogen or C_1-C_4 alkyl and the other is C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy; or R and R_1 are the same and are C_1-C_4 alkoxy.

Pharmaceutically acceptable salts of the compounds of formula (I) are their salts with pharmaceutically acceptable, either inorganic or organic, acids.

Examples of inorganic acids are hydrochloric, hydrobromic, sulfuric and nitric acid; examples of organic acids are acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic and p-toluenesulfonic acid.

A particularly preferred n value is 3.

X is preferably chloro or bromo, in particular chloro.

A preferred class of compounds according to the present invention are the compounds of formula (I) wherein:

n is 3;

X is chloro;

one of R and R_1 is hydrogen or C_1-C_4 alkyl and the other is C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy; and the pharmaceutically acceptable salt thereof.

Examples of specific compounds under this invention, especially in the form of salts preferably with hydrochloric acid, are the following:

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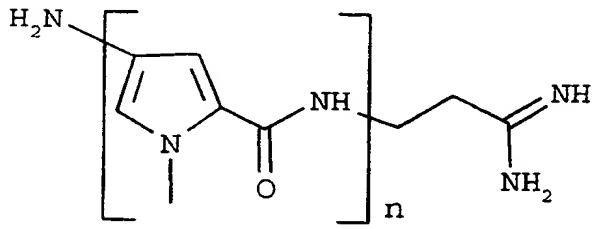
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine; and

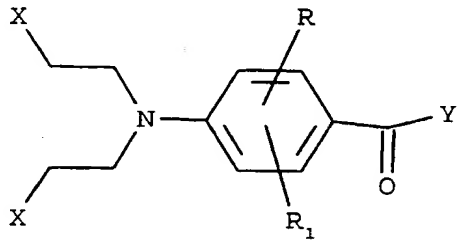
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-5-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido] pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine.

The compounds of the invention and the salts thereof can be obtained by a process comprising reacting a compound of formula (II)



(II)

wherein n is as defined above, with a compound of formula (III)



(III)

wherein

R, R_1 and X are as defined above and Y is hydroxy or leaving group; and, if desired, salifying a compound of formula (I) or obtaining a free compound from a salt thereof, and/or, if desired, separating a mixture of isomers of a compound of formula (I) into the single isomers.

The reaction of a compound of formula (II) with a compound of formula (III) can be carried out according to known methods,

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by known standard methods.

Well known procedures such as, e.g. fractional crystallization or chromatography may also be followed for separating a mixture of isomers of formula (I) into the single isomers.

The new compounds of formula (I) prepared according to the above described procedures may be as well purified by conventional methods such as, e.g., silica gel or alumina column chromatography, and/or by recrystallization from an organic solvent such as, e.g., a lower aliphatic alcohol, e.g. methyl, ethyl or isopropyl alcohol, or dimethylformamide.

PHARMACOLOGY

The compounds of the invention can be useful as antineoplastic and antiviral agents. They show, in particular, cytostatic properties towards tumor cells so that they can be useful, e.g., to inhibit the growth of various tumors, such as, for instance, carcinomas, e.g. mammary carcinoma, lung carcinoma, bladder carcinoma, colon carcinoma, ovary and endometrial tumors in mammals, including humans. Other neoplasias in which the compounds of the invention could find application are, for instance, sarcomas, e.g. soft tissue and bone sarcomas, and the hematological malignancies such as, e.g. leukemias.

The antitumor activity was evaluated in vitro by cytotoxicity studies carried out on murine L1210 leukemia cell. Cells were derived from in vivo tumors and established in cell culture. Cells were used until the tenth passage. Cytotoxicity was determined by counting surviving cells after 48 hours treatment.

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The compounds of the invention showed higher antitumor activity in these tumor models than closely related compounds known from EP-B-0246868.

For example, the representative compounds β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl) aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine (internal code FCE 29325) and β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-dimethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] pyrrole-2-carboxamido]propionamidine (internal code FCE 29721) and the prior art compound, according to EP-B-0246868, β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido] pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine (internal code FCE 24517), were tested against disseminated L_{1210} murine leukemia showing the following activity data.

Table 1

Compound (internal code)	*mg/kg*	*T/C %*	*Tox*
FCE 29325	3.13	191	0/10
FCE 29721	3.13	183	0/10
FCE 24517	3.13	133	0/10

The activity data occurring in above Table 1 show that the compounds of the instant invention, bearing the claimed substituents on the phenyl ring of the benzoyl mustard moiety, are more active than the closely related unsubstituted prior art compound FCE 24517.

The compounds of the invention show also a remarkable effectiveness in interfering with the reproductive activity

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As already said, the pharmaceutical compositions of the invention contain a compound of formula (I) as the active substance, in association with one or more pharmaceutically acceptable excipients.

The pharmaceutical compositions of the invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form.

For instance, solutions for intravenous injection of infusion may contain as carrier, for example, sterile water or preferably, they may be in the form of sterile aqueous isotonic saline solutions.

Suspensions or solutions for intramuscular injections may contain, together with the active compound a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

In the forms for topical application, e.g. creams, lotions or pastes for use in dermatological treatment, the active ingredient may be mixed with conventional oleaginous or emulsifying excipients.

The solid oral forms, e.g. tablets and capsules, may contain, together with the active compound, diluents, e.g., lactose, dextrose, saccharose, cellulose, corn starch and potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents, e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethyl cellulose, polyvinylpyrrolidone; disaggregating agents, e.g. a starch, alginic acid, alginates, sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, for instance, lecithin, polysorbates, laurylsulphates; and,

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doxorubicin, daunomycin, epirubicin, idarubicin, etoposide, fluoro-uracil, melphalan, cyclophosphamide, 4-demethoxy daunorubicin, bleomycin, vinblastin and mitomycin or a mixtures of two or more thereof.

The compounds of the invention can therefore be used in a treatment to ameliorate a cancer. They may be administered to a patient suffering from a cancer treatable with an antitumor agent, for example an anthracycline glycoside such as doxorubicin, daunomycin, epirubicin, 4-demethoxy daunorubicin or idarubicin as mentioned above, together with the antitumor agent.

A compound of the invention and an antitumor agent such as an anthracycline glycoside can be administered to improve the condition of a patient having a leukaemia lymphoma, sarcoma, such as myeloblastic leukaemia, neuroblastoma, Wilm's tumor or malignant neoplasm of the bladder, breast, lung or thyroid.

The following examples illustrate but do not limit the invention.

The abbreviations DMF, DMSO and P.M.R. stand for dimethylformamide, dimethylsulfoxide and proton magnetic resonance respectively.

Example 1

The compound β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine hydrochloride

The mixture was stirred for one hour and then added of a solution of hydrochloric acid 2N until pH=1. The solvent was evaporated in vacuo and the solid residue purified by flash chromatography on silica gel with a mixture of methylene chloride, methanol, yielding 500 mg of the title compound.

FAB-MS: m/z: 711 (45[M+H]$^+$), 258 (75)

P.M.R. (DMSO) δ: 10.19 (s, 1H); 9.97 (s, 1H); 9.91 (s, 1H); 8.7 (bs, 4H); 8.21 (t, J=5.7 Hz, 1H); 7.74 (m, 2H); 7.29 (d, J=1.8 Hz, 1H); 7.28 (d, J=7.5 Hz, 1H); 7.22 (d, J=1.8 Hz, 1H); 7.17 (d, J=1.8 Hz, 1H); 7.08 (d, J=1.8 Hz, 1H); 7.05 (d, J=1.8 Hz, 1H); 6.94 (d, J=1.8 Hz, 1H); 3.85 (s, 3H); 3.83 (s, 3H); 3.80 (s, 3H); 3.3-3.7 (m, 10H); 2.6 (t, J=6.6 Hz, 2H); 2.33 (s, 3H).

By analogous procedure and using the opportune intermediate the following compounds can be obtained:

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-dimethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine hydrochloride

FAB-MS: m/z: 725 (90[M+H]$^+$)

U.V. (EtOH 95%) λ 310; ε = 42985

P.M.R. (DMSO) δ: 10.22 (s, 1H); 10.01 (s, 1H); 9.94 (s, 1H); 8.99 (s, 2H); 8.64 (s, 2H); 8.21 (t, J=5.7 Hz, 1H); 7.61 (s, 2H); 7.29 (d, J=1.7 Hz, 1H); 7.21 (d, J=1.7 Hz, 1H); 7.18 (d, J=1.7 Hz, 1H); 7.08 (d, J=1.7 Hz, 1H); 7.05 (d, J=1.7 Hz, 1H); 6.91 (d, J=1.7 Hz, 1H); 3.86 (s, 3H); 3.84 (s, 3H); 3.81 (s, 3H); 3.62 (m, 2H); 3.60-3.30 (m, 8H); 2.62 (m, 2H); 2.35 (s, 6H).

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methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido] pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine.

Example 2

Tablets each weighting 0.250 g and containing 50 mg of the active substance can be manufactured as follows:

Composition for 10.000 tablets	
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido] pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride	500 g
Lactose	1.400 g
Corn starch	500 g
Talc powder	80 g
Magnesium stearate	20 g

The β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis (2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine hydrochloride, the lactose and half the corn starch are mixed; the mixture is then forced through a sieve of 0.5 mm mesh size.

Corn starch (10 g) is suspended in warm water (90 ml) and the resulting paste is used to granulate the powder. The granulate is dried, comminuted on a sieve of 1.4 mm mesh size, then the remaining quantity of starch, talc and magnesium stearate is added, carefully mixed and processed into tablets.

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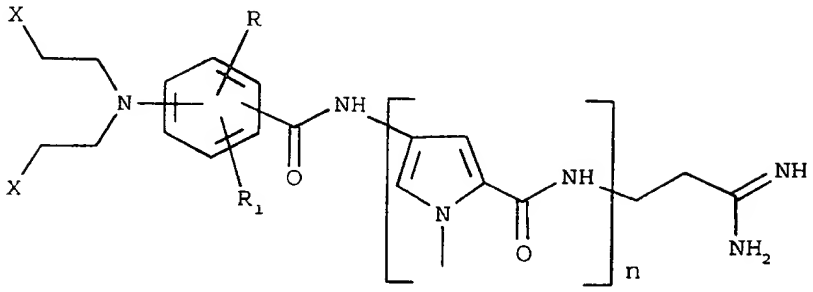
CLAIMS

1. A compound of formula (I)



(I)

wherein

n is 2, 3 or 4;

one of R and R_1 is hydrogen, C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy and the other is independently CF_3, C_1-C_4 alkyl or C_1-C_4 alkoxy; and

X is halogen and the pharmaceutically acceptable salts thereof.

2. A compound of formula (I), according to claim 1, wherein

n is 3;

X is chloro;

one of R and R_1 is hydrogen or C_1-C_4 alkyl and the other is C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy; and the pharmaceutically acceptable salts thereof.

3. A compound selected from:

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]

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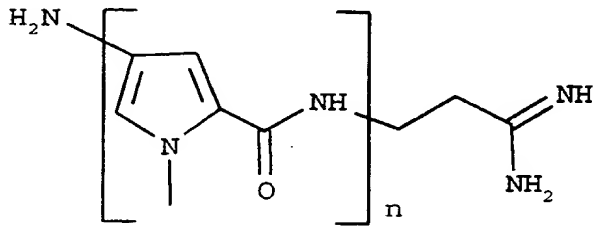
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-5-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine; or a pharmaceutically acceptable salt thereof.

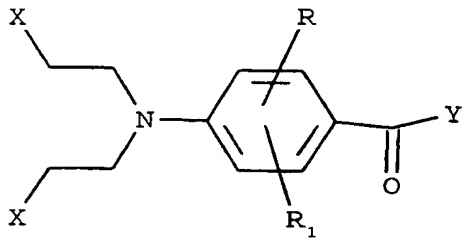
4. A salt of a compound according to claim 3, wherein said salt is the hydrochloride.

5. A process for the preparation of a compound of formula (I), according to claim 1, or a salt thereof, said process comprising reacting a compound of formula (II)



(II)

wherein n is as defined in claim 1, with a compound of formula (III)



(III)

wherein

R, R_1 and X are as defined in claim 1 and Y is hydroxy or leaving group; and, if desired, salifying a compound of formula (I) or obtaining a free compound from a salt thereof, and/or, if desired, separating a mixture of isomers of a compound of formula (I) into the single isomers.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/02659

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D207/34 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. MED. CHEM. (1989), 32(4), 774-8 CODEN: JMCMAR;ISSN: 0022-2623, 1989, XP000608784 ARCAMONE, FEDERICO MARIA ET AL: "Synthesis, DNA-binding properties, and antitumor activity of novel distamycin derivatives" see the whole document ---	1-10
A	EP 0 246 868 A (FARMITALIA CARLO ERBA S.P.A., ITALY) 25 November 1987 cited in the application see the whole document -----	1-10

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 1996	19.11.1996

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Kissler, B

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 96/02659

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0246868	25-11-87	AU-B-	597659	07-06-90
		AU-A-	7316387	26-11-87
		BG-B-	60531	28-07-95
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		HK-A-	31993	08-04-93
		IE-B-	60198	15-06-94
		JP-B-	6023193	30-03-94
		JP-A-	62294653	22-12-87
		KR-B-	9511408	04-10-95
		SU-A-	1528316	07-12-89
		US-A-	5017599	21-05-91
		US-A-	5049579	17-09-91
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		ZA-A-	8703593	12-11-87

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